Developmental Biology **244,** 1–8 (2002)
doi:10.1006/dbio.2001.0566, available online at http://www.idealibrary.com on IDEAL®

TCF Transcription Factors, Mediators of Wnt-Signaling in Development and Cancer

Mascha van Noort and Hans Clevers[1]

Department of Immunology, UMC Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

The Wnt-signaling cascade plays critical roles in embryonic patterning, cell fate determination, and tissue homeostasis. When secreted Wnt factors bind to their receptors, a set of biochemical changes is set in motion, ultimately resulting in the accumulation of β-catenin and its translocation to the nucleus. In the nucleus, β-catenin binds to members of the TCF/LEF family of transcription factors, the most downstream components of the Wnt-signaling pathway. Here, an overview is given of this tightly regulated cascade, with particular emphasis on events that take place in the nucleus.

THE TCF/LEF FAMILY OF TRANSCRIPTION FACTORS

The founding members of the TCF/LEF family of transcription factors, TCF-1 and LEF-1, were identified in screens for T cell-specific transcription factors. TCF-1 was identified by its ability to bind to the *CD3ε* enhancer, whereas LEF-1 was found in a screen for proteins binding to the *TCRα* enhancer and a site in the HIV LTR (Oosterwegel *et al.*, 1991; van de Wetering *et al.*, 1991; Travis *et al.*, 1991; Waterman *et al.*, 1991). In more recent years, two additional family members were identified in mammals: *TCF-3* and *TCF-4* (Korinek *et al.*, 1998a). The *Drosophila* genome only contains one TCF gene, called *dtcf* or *pangolin* (Brunner *et al.*, 1997; van de Wetering *et al.*, 1997). Similarly, a single gene resides in the genome of the nematode *Caenorhabditis elegans*, *Pop-1* (Lin *et al.*, 1995).

Proteins of the TCF/LEF family contain an 80-amino-acid high mobility group (HMG) box. HMG boxes bind DNA as monomers and can do so (Grosschedl *et al.*, 1994; Laudet *et al.*, 1993) in a sequence-specific manner (Laudet *et al.*, 1993). The TCF consensus recognition sequence is remarkably conserved between the family members and comprises AGATCAAAGGG (van de Wetering *et al.*, 1991; Giese *et al.*, 1991; van Beest *et al.*, 2000). The HMG box not only mediates DNA sequence recognition, but coincidently induces a dramatic bend in the DNA (Giese *et al.*, 1992; Dooijes *et al.*, 1993). By doing so, HMG boxes may coordinate the binding of other transcription factors (Grosschedl *et al.*, 1994; Giese *et al.*, 1995; Bianchi and Beltrame, 1998). Because of this bending ability and the observation that TCF factors cannot directly activate transcription in reporter assays, it has been proposed that TCF/LEF family members primarily serve an architectural function. LEF-1 appears unique in that it contains a context-dependent activation domain (CAD) (Carlsson *et al.*, 1993), which can activate transcription in the presence of the coactivator ALY (Bruhn *et al.*, 1997). The other TCF family members do not appear to contain a CAD domain (van de Wetering *et al.*, 1996).

TCF/Lef mRNAs undergo extensive alternative splicing. The best studied gene, TCF-1, also exhibits alternative promoter usage. Over 100 Tcf-1 isoforms may theoretically be produced. However, Western blotting has revealed the predominant expression of eight different TCF-1 protein isoforms (van de Wetering *et al.*, 1996). LEF-1 exhibits two splice variants (Waterman *et al.*, 1991) and also possesses two promoters (Hovanes *et al.*, 2001). Several C-terminal splice variants exist of TCF-4 that exhibit strong sequence homology to similar TCF-1 mRNAs (Korinek *et al.*, 1997; Duval *et al.*, 2000) (Fig. 1). While the functional relevance of the alternative splice products in TCF/Lef genes remains unclear at present, alternative promoter usage in the TCF-1 and LEF-1 gene generates protein isoforms that either carry or lack the N-terminal β-catenin interaction domain (see below).

TCF/LEF KNOCKOUT MICE

The molecular context in which the TCF family of transcription factors functions remained mysterious for years. Knockout mice have been generated for three murine Tcfs. In adult animals, Tcf-1 is expressed in T lymphocytes. Indeed, *Tcf-1*$^{-/-}$ knockout mice are impaired in the generation of T lymphocytes (Verbeek *et al.*, 1995). Their thymuses are reduced in size and show a 10- to 100-fold

[1] To whom correspondence should be addressed. Fax: +31 (30) 2517107. E-mail: h.clevers@azu.nl.

0012-1606/02 $35.00

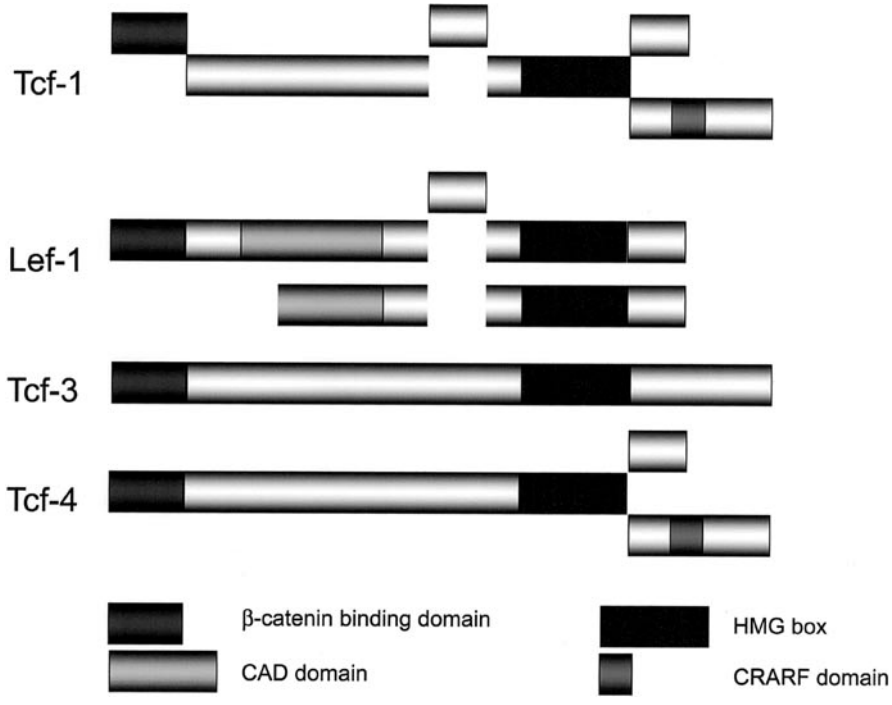

FIG. 1. Schematic representation of Tcf splice variants and their most conserved domains. Short forms of Tcf-1 lack the N-terminal domain, which interacts with β-catenin. The most distinct regions of the Tcf family members is the C terminus, which contains the CRARF domain in the longer versions.

reduction in T cell numbers compared with heterozygous littermates (Verbeek *et al.*, 1995). Nevertheless, the *Tcf-1*$^{-/-}$ mice are fully immunocompetent, have functional peripheral T cells, have a normal lifespan, and are fertile (Schilham *et al.*, 1998). Closer examination of the thymocytes from 4- to 6-week-old animals revealed proliferative abnormalities at several precursor stages, which are normally characterized by extensive cell expansion (Schilham *et al.*, 1998). However, mature T cells as present in spleen and lymph nodes are fully competent upon encountering mitogens or antigens. Tcf-1 thus appears essential for the maintenance of early thymocyte progenitors.

More recently, it was found that *Tcf-1*$^{-/-}$ mice develop adenomas in the gut and the mammary glands. This led to the hypothesis that Tcf-1 is a target gene of Tcf-4/β-catenin signaling in the intestine (Roose *et al.*, 1999). The predominantly expressed isoform of Tcf-1 in the gut lacks the N-terminal β-catenin interaction domain and may function in a negative feedback loop.

Lef-1$^{-/-}$ knockout mice have a more complex phenotype. During embryogenesis, Lef-1 is expressed at many different sites, including the tel-, di-, and mesencephalon, tooth germs, whisker follikels, mammary buds, thymus, limb buds, and tail vertebrae (Oosterwegel *et al.*, 1993; Zhou *et al.*, 1995; van Genderen *et al.*, 1994). Mice homozygous for a mutation in the Lef gene die shortly after birth and show deficiencies in some but not all organs normally expressing Lef-1 (van Genderen *et al.*, 1994). Most strikingly, these mice lack body hair and whiskers. Although follicle formation starts at the proper time point, only one-third of normal hair follicle numbers is found later in development. These hair follicles are short and rudimentary. These *Lef-1*$^{-/-}$ mice also lack teeth. The tooth germs are indistinguishable from wild-type counterparts at the bud stage (E13). Yet, normal progression of the *Lef-1*$^{-/-}$ buds to the late cap stage (E15) is halted (van Genderen *et al.*, 1994). Newborn *Lef1*$^{-/-}$ females reveal a stunted development of mammary glands. The mutant mice also lack the trigeminal nucleus. Unexpectedly, the mice did not show any obvious defects in lymphoid cell populations at birth, despite the fact that *Lef-1* is expressed in B-cells (van Genderen *et al.*, 1994). Nevertheless, it was shown in a recent study that Lef-1 mediates proliferation of early B lineage cells *in vitro* (Reya *et al.*, 2000).

Tcf-1/Lef-1 double knockout mice made with a hypomorphic Tcf-1 allele display a very severe defect in T cell differentiation, but no developmental defects. α/β T cell differentiation is completely blocked at the immature single positive (ISP) stage and clearly impaired at earlier stages (Okamura *et al.*, 1998). Crossing a null-allele of *Tcf-1* into *Lef-1* knockout mice yields an early embryonic phenotype that resembles that of *Wnt-3A*. Severe defects occur in the differentiation of the paraxial mesoderm, resulting in the formation of additional neural tubes. These embryos

also show defects in placenta formation and in the formation of the apical ectodermal ridge of the limb buds. These data indicate extensive functional redundancy between Tcf-1 and Lef-1 in early T lymphocytes and during embryonic development (Galceran *et al.*, 1999).

Tcf-4 is expressed from E10.5 onward in the developing CNS, where it partially overlaps with *Lef-1* expression. Tcf-4 expression is particularly striking in gut epithelium throughout life (Korinek *et al.*, 1997, 1998a). *Tcf-4*$^{-/-}$ mice die shortly after birth and display a single phenotypic abnormality, the complete absence of the stem cell compartment in the crypts of the small intestine (Korinek *et al.*, 1998b). The organization of the pseudostratified epithelium in the small intestine of mutant embryos shows no aberrations at day 14.5 compared with control littermates. At day 16.5, however, a reduced number of cells populates the prospective crypt region and the number of villi is reduced (Korinek *et al.*, 1998b). Closer examination of the cells populating the prospective crypt regions reveals a lack of actively dividing crypt cells, implying that Tcf-4, like Tcf-1, functions to maintain early progenitor cells.

BINDING PARTNERS OF TCF

The identification of binding partners of TCF/LEF eventually revealed their molecular context. The first protein found to interact with TCF/LEF factors was the Wnt signaling effector β-catenin (Molenaar *et al.*, 1996; Behrens *et al.*, 1996). Using multimerized TCF binding motifs upstream of a reporter gene, it became apparent that cotransfection of TCF with β-catenin strongly induced transcription (Molenaar *et al.*, 1996). All members of the TCF family can bind β-catenin through a conserved N-terminal stretch of 55 amino acids. β-Catenin thus functions as a classical coactivator of transcription. A potent transcriptional activation domain maps to its C terminus. Recent studies have indicated that this domain serves to modify local chromatin. The histone acetylase CBP was found to interact directly with the transactivation domain of β-catenin and synergizes with the latter protein to enhance transcription of a reporter gene *in vivo* (Takemaru and Moon, 2000). Similarly, the C-terminal transactivation domain of β-catenin binds to Brg-1, a core component of the mammalian Swi/Snf chromatin remodeling complex. Binding of Brg-1 to β-catenin enhances transcription of Tcf target genes (Barker *et al.*, 2001).

Another binding partner of TCF, Grg-5, is related to *Drosophila* Groucho, which in turn is the founding member of a family of broadly expressed transcriptional corepressors. Grg proteins interact with histone deacetylase-1 (HDAC) and can thus mediate the condensation of chromatin (Chen *et al.*, 1999). In a transient reporter assay, the transcription driven by β-catenin/TCF could completely be repressed by coexpression of Grg expression constructs (Roose *et al.*, 1998; Cavallo *et al.*, 1998). In mammals, all members of the TCF family can bind to all Grgs; the combinations appear to be equally potent in repressing transcription (Brantjes *et al.*, 2001). The shorter Grg family member, Grg-5, fails to bind to HDAC, acting thereby as a derepressor (Roose *et al.*, 1998; Brantjes *et al.*, 2001).

In Drososphila, another repressor of β-catenin/TCF was identified as CBP (Waltzer and Bienz, 1998). This was rather surprising, as CBP is a strong coactivator for dozens of other transcription factors, including β-catenin. CBP binds to the HMG box and subsequently acetylates a specific lysine in the N-terminal β-catenin interaction domain. This leads to decreased binding of β-catenin to TCF, resulting in lower transactivation. The lysine to be acetylated, K25, is absent from the N termini of TCF-1, TCF-3, and the worm POP-1. This suggests that this mode of modulating β-catenin/Tcf-mediated signaling may be dependent on the identity of the TCF/Lef family member. The phenotype of the dCBP mutant embryos suggests that this activity of dCBP primarily antagonizes Wnt signaling in cells which are only stimulated weakly (Waltzer and Bienz, 1998). Finally, another protein found to function as corepressor for a subset of the TCF family was described by Moon and coworkers (Brannon *et al.*, 1999). C-terminal binding protein (CtBP) binds to a sequence only present in the C terminus of XTcf-3 and TCF-4, thereby converting these TCF proteins into repressors.

Taken together, TCFs act as repressors in the absence of Wnt signaling (see below) by complex formation with one of several corepressors. Transcriptional repression involves histone deacetylation, but may utilize other yet unidentified mechanisms. Upon the delivery of a Wnt signal, complex formation with β-catenin recruits histone acetylases (CBP) and chromatin remodelers (Brg1) to TCF target genes to transiently activate their transcription.

TCF AND WNT-SIGNALING

The identification of β-catenin as a binding partner of TCF answered two questions at once. It provided a mechanism by which TCF could regulate transcription, and also revealed the nature of the final step in the Wnt cascade. β-Catenin was the most downstream protein in the Wnt-signaling cascade known at the time, but it was unclear how β-catenin was able to transduce the signal into the nucleus, as β-catenin has no DNA-binding properties. TCF, on the other hand, was known to bind to DNA, but by itself did not regulate transcription. Now that β-catenin and TCF were found to interact, TCF could be placed downstream of β-catenin in the Wnt-signaling cascade.

THE CORE OF THE WNT PATHWAY

The Wnt/wingless proteins constitute a large family of cysteine-rich glycoproteins. Wnts function as ligands for members of the Frizzled (Fz) family of serpentine receptors (Bhanot *et al.*, 1996). Low-density lipoprotein (LDL)

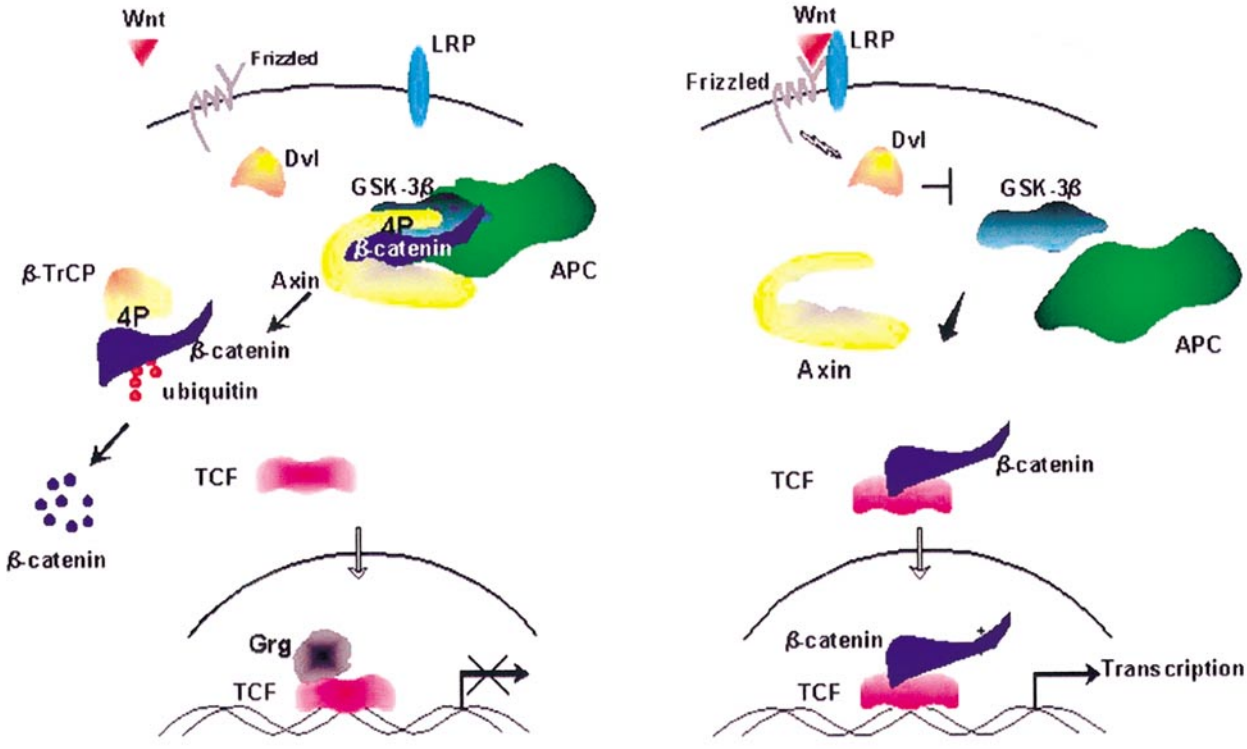

FIG. 2. Model of the Wnt signaling cascade. This simplified scheme depicts the differences between a nonsignaling cell and its signaling counterpart.

receptor-related proteins (LRPs) act in synergy with Fz. The extracellular domains of both proteins interact, and signaling of Wnt is only mediated when both Fz and LRP are complexed with Wnt-1 (Tamai *et al.*, 2000; Wehrli *et al.*, 2000; Pinson *et al.*, 2000).

In unstimulated cells, β-catenin resides in a large cytoplasmic complex consisting of the tumor suppressor APC (adenomatous polyposis coli), GSK-3β (glycogen synthase kinase 3β), and Axin (Behrens *et al.*, 1998; Kishida *et al.*, 1998; Ikeda *et al.*, 1998). In this destruction complex, β-catenin is phosphorylated by the serine/threonine kinase GSK-3β on four N-terminal serine and threonine residues. The phosphorylated residues are recognized by β-TrCP, which resides in an E3 ubiquitin ligase complex. Ubiquitination of β-catenin induces its rapid proteasomal degradation (Orford *et al.*, 1997). Upon Wnt binding to its receptors, the Wnt-signaling cascade is activated (Fig. 2). This results in inhibition of the constitutive activity of GSK-3β (Siegfried *et al.*, 1994; Dominguez *et al.*, 1995; He *et al.*, 1995; Cook *et al.*, 1996). Consequently, β-catenin is no longer phosphorylated and can accumulate in the cytoplasm and nucleus (Funayama *et al.*, 1995; Schneider *et al.*, 1996; Yost *et al.*, 1996). Nuclear β-catenin interacts with members of the TCF family, resulting in target gene transcription (Molenaar *et al.*, 1996; Brunner *et al.*, 1997; Riese *et al.*, 1997; van de Wetering *et al.*, 1997). (For a more detailed description of the Wnt signaling cascade see reviews in Wodarz and Nusse, 1998; Miller *et al.*, 1999; Polakis, 2000; Peifer and Polakis, 2000.)

The involvement of TCF/LEF in the Wnt-signaling pathway was first addressed in *Xenopus laevis*. The role of β-catenin in dorsal axis formation had been known for years (Heasman *et al.*, 1994). Ventral microinjection of *β-catenin* mRNA in embryos induces axis duplication. This phenotype could be prevented by coinjecting mRNA encoding an XTcf-3 protein lacking the β-catenin binding domain. Dorsal injection of this Tcf mutant could also block endogenous head induction (Molenaar *et al.*, 1996). Injections of *Lef-1* mRNA lacking the HMG box also inhibited head induction (Behrens *et al.*, 1996; Huber *et al.*, 1996). These data proved that the direct interaction of β-catenin with TCF/LEF is essential for normal axis specification in *Xenopus* and tentatively placed TCF/LEF directly downstream of β-catenin in the Wnt cascade.

Since the experiments in Xenopus largely relied on overexpression of proteins, definitive proof of the role of Tcf in the Wnt signaling cascade was subsequently sought from genetic experiments in *Drosophila*. Two labs independently cloned the fly homologue of Tcf, *dTcf* or *pangolin* (van de Wetering *et al.*, 1997; Brunner *et al.*, 1997). dTcf binds a Tcf DNA motif and, together with the fly β-catenin homologue *armadillo*, transactivates transcription of reporter genes.

The isolation of dTcf-mutant flies revealed segment polarity phenotypes as expected for Wnt pathway mutants. Loss-of-function mutations in *dTcf* were dominant over gain-of-function mutations in *armadillo* (van de Wetering *et al.,* 1997), confirming the observations in *X. laevis* (see above). Moreover, murine *Lef-1* overexpression in transgenic flies was found to mimic hyperstimulation of the Wnt pathway (Bruhn *et al.,* 1997).

Studies in the nematode *C. elegans* on the role of β-catenin and TCF were complicated for two reasons. First, at least two different Wnt signal transduction cascades exist. Second, the *C. elegans* genome harbors three β-catenin homologues: WRM-1, BAR-1, and HMP-2. Wnt signaling was originally studied in the specification of the endoderm (E) and mesoderm (MS) cells from a common precursor (EMS) at the four- to eight-stage. In this pathway, the β-catenin homologue WRM-1 unexpectedly opposes the activity of the Tcf homologue POP-1. This activity requires the formation of a ternary complex with the MAP kinase-like LIT-1 protein (Lin *et al.,* 1995; Rocheleau *et al.,* 1999). Reinterpretation of these data suggests that, in the EMS Wnt pathway, the Tcf homologue POP-1 only acts as a repressor. Activation of LIT-1/WRM1 by Wnt signaling results in the inactivation of the repressive POP-1. This alternative Wnt pathway may also exist in mammals (Ishitani *et al.,* 1999).

More recently published data demonstrate that a canonical Wnt signaling pathway does exist in *C. elegans*. The pathway utilizes one of the other β-catenin homologs, BAR-1, in conjunction with the Tcf homologue POP-1. BAR-1 acts like β-catenin in activating target genes in response to Wnt signals. Developmental events controlled by BAR-1/POP-1 in this canonical Wnt pathway include vulva development and migration of a specific neuron, the Q cell, and its offspring (Korswagen *et al.,* 2000).

MUTATIONAL ACTIVATION OF TCF IN CANCER

Wnt-1 was first identified by retroviral integration in mammary tumors in mice, implying that Wnt-signaling plays a role in tumor development. Wnts do not appear to play a dominant role in human cancer. Rather, mutations in downstream components of the Wnt cascade are a major cause of several types of cancer. APC is mutated at both alleles in 80% of all colon carcinomas (Polakis *et al.,* 1999). These mutations almost invariably lead to a truncation of APC, removing its interaction domains for β-catenin and axin. As a consequence, the activity of the destruction complex is impaired and cytosolic β-catenin is no longer phosphorylated and degraded, but rather accumulates. The accumulated β-catenin in turn inappropriately activates one of the TCF/LEF family members that is specifically expressed in the intestinal epithelium, TCF-4 (Korinek *et al.,* 1997). In a significant fraction of sporadic colon tumors lacking *APC* mutations, mutations were found in the *CTNNB1* (β-catenin) gene. These mutations occur in or around the third exon, which encodes the putative phosphorylation sites of GSK-3β and remove the target residues of this kinase (Morin *et al.,* 1997; Polakis *et al.,* 1999). Mutations in β-catenin are not only found in intestinal tumors. The *CTNNB1* gene has been sequenced extensively in various other tumors. An overview of the mutations found in β-catenin can be found at the Wnt-signaling website (www.ana.ed.ac.uk/rnusse/pathways/bcatmut.html). As the exception that proves the rule, rare mutations in Axin/Conduction have also been found in colon tumors and other types of malignancies (Webster *et al.,* 2000).

Taken together, mutations in APC, axin, and β-catenin share a common denominator, in that they all lead to the formation of β-catenin/TCF complexes. This implies that the inappropriate activation of TCF target genes in epithelial cells represents the primary event in cellular transformation (reviewed in Polakis, 2000; Fodde and Smits, 2001).

THE BIOLOGY OF INTESTINAL CRYPT CELLS

Why would the inappropriate activation of TCF provide such a potent transforming signal? These data imply an important role for TCF-4 in the physiology of the intestinal epithelium. This epithelium consists of alternating crypts and villi (Potten and Loeffler, 1990). The proliferating stem cell population is located in the crypts, from which cells slowly migrate up toward the villus. These cells differentiate into various distinct cell types. Once reaching the apex of the villus, the cells die and are shed into the gut lumen. A Wnt signal activates Tcf-4 to instruct epithelial cells to adopt a stem cell phenotype. It is obvious how the inappropriate activation of Tcf-4 would result in the unrestrained proliferation of undifferentiated cells. Abnormal outpocketing of the intestinal epithelium at the crypt-villus border is the first sign of polyp formation. This pocket protrudes into the inside of the neighboring villus, subsequently filling the total space. APC loss and the subsequent stabilization of β-catenin results in an increase in size of the proliferative compartment in the crypt region. As Tcf4 expression is required for crypt maintenance, it is likely that inappropriate Tcf4/β-catenin signaling is responsible for this phenomenon. Cells that normally would start to differentiate when migrating up the crypt-villus axis now are kept in a proliferative and nondifferentiated state; in other words, they remain crypt cells. This is supported by the fact that β-catenin has been observed to suppress differentiation (Harada *et al.,* 1999).

A number of TCF target genes have been proposed to play a role in the transformation of intestinal epithelial cells. These include *c-MYC* (He *et al.,* 1998), *cyclin D1* (Tetsu and McCormick, 1999), *TCF-1* (Roose *et al.,* 1999), *gastrin* (Koh *et al.,* 2000), and *PPARδ* (He *et al.,* 1999). For a complete list visit the Wnt website: www.ana.ed.ac.uk/rnusse/wntwindow.html. Although these target genes may play a role in

cellular transformation, these studies provide only snapshots of the entire TCF target gene program in colorectal cancer. Global insights into this genetic program will undoubtedly be generated in the near future through the application of DNA array analysis.

The detailed molecular understanding of the Wnt-signaling cascade now allows the search for drugs that selectively target specific steps of the pathway. Structures of the β-catenin/Tcf3 and β-catenin/E-cadherin complexes have recently been elucidated (Graham *et al.*, 2000; Huber and Weis, 2001). Birchmeier and colleagues have performed an extensive mutational analysis of the basic groove that runs along the length of the armadillo-repeat region of β-catenin (von Kries *et al.*, 2000). This groove binds Tcf, E-cadherin, and APC in a mutually exclusive fashion. In addition, it binds axin. This study revealed unique hotspots in the groove for each of these four interaction partners. This has raised hopes that unique inhibitors can be designed for the β-catenin/Tcf interaction. Such inhibitors may constitute highly precise drugs for the treatment of colorectal cancer.

ACKNOWLEDGMENTS

We thank Dr. Rachel Giles for her valuable advice on the manuscript, as well as the members of the Clevers lab for helpful discussions.

REFERENCES

Barker, N., Hurlstone, A., Musisi, H., Miles, A., Bienz, M., and Clevers, H. (2001). The chromatin remodelling factor Brg-1 interacts with beta-catenin to promote target gene activation. *EMBO J.* **20,** 4935–4943.

Behrens, J., Jerchow, B. A., Wurtele, M., Grimm, J., Asbrand, C., Wirtz, R., Kuhl, M., Wedlich, D., and Birchmeier, W. (1998). Functional interaction of an axin homolog, conduction, with beta-catenin, APC, and GSK3beta. *Science* **280,** 596–599.

Behrens, J., von Kries, J. P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. (1996). Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* **382,** 638–642.

Bhanot, P., Brink, M., Samos, C. H., Hsieh, J. C., Wang, Y., Macke, J. P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from Drosophila functions as a Wingless receptor. *Nature* **382,** 225–230.

Bianchi, M. E., and Beltrame, M. (1998). Flexing DNA: HMG-box proteins and their partners. *Am. J. Hum. Genet.* **63,** 1573–1577.

Brannon, M., Brown, J. D., Bates, R., Kimelman, D., and Moon, R. T. (1999). XCtBP is a XTcf-3 co-repressor with roles throughout Xenopus development. *Development* **126,** 3159–3170.

Brantjes, H., Roose, J., van de Wetering, M., and Clevers, H. (2001). All Tcf HMG box transcription factors interact with Groucho-related co-repressors. *Nucleic Acids Res.* **29,** 1410–1419.

Bruhn, L., Munnerlyn, A., and Grosschedl, R. (1997). ALY, a context-dependent coactivator of LEF-1 and AML-1, is required for TCRalpha enhancer function. *Genes Dev.* **11,** 640–653.

Brunner, E., Peter, O., Schweizer, L., and Basler, K. (1997). pangolin encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the Wingless signal in Drosophila. *Nature* **385,** 829–833.

Carlsson, P., Waterman, M. L., and Jones, K. A. (1993). The hLEF/TCF-1 alpha HMG protein contains a context-dependent transcriptional activation domain that induces the TCR alpha enhancer in T cells. *Genes Dev.* **7,** 2418–2430.

Cavallo, R., Cox, R., Moline, M., Roose, J., Polevoy, G., Clevers, H., Peifer, M., and Bejsovec, A. (1998). *Drosophila* Tcf and Groucho interact to repress Wingless signalling activity. *Nature* **395,** 604–608.

Chen, G., Fernandez, J., Mische, S., and Courey, A. J. (1999). A functional interaction between the histone deacetylase Rpd3 and the corepressor groucho in Drosophila development. *Genes Dev.* **13,** 2218–2230.

Cook, D., Fry, M. J., Hughes, K., Sumathipala, R., Woodgett, J. R., and Dale, T. C. (1996). Wingless inactivates glycogen synthase kinase-3 via an intracellular signalling pathway which involves a protein kinase C. *EMBO J.* **15,** 4526–4536.

Dominguez, I., Itoh, K., and Sokol, S. Y. (1995). Role of glycogen synthase kinase 3 beta as a negative regulator of dorsoventral axis formation in Xenopus embryos. *Proc. Natl. Acad. Sci. USA* **92,** 8498–8502.

Dooijes, D., van de, W. M., Knippels, L., and Clevers, H. (1993). The Schizosaccharomyces pombe mating-type gene mat-Mc encodes a sequence-specific DNA-binding high mobility group box protein. *J. Biol. Chem.* **268,** 24813–24817.

Duval, A., Rolland, S., Tubacher, E., Bui, H., Thomas, G., and Hamelin, R. (2000). The human T-cell transcription factor-4 gene: Structure, extensive characterization of alternative splicings, and mutational analysis in colorectal cancer cell lines. *Cancer Res.* **60,** 3872–3879.

Fodde, R., Kuipers, J., Rosenberg, C., Smits, R., Kielman, M., Gaspar, C., van Es, J. H., Breukel, C., Wiegant, J., Giles, R. H., and Clevers, H. (2001). Mutations in the APC tumour suppressor gene cause chromosomal instability. *Nat. Cell Biol.* **3,** 433–438.

Fodde, R., and Smits, R. (2001). Disease model: Familial adenomatous polyposis. *Trends Mol. Med.* **7,** 369–373.

Funayama, N., Fagotto, F., McCrea, P., and Gumbiner, B. M. (1995). Embryonic axis induction by the armadillo repeat domain of beta-catenin: Evidence for intracellular signaling. *J. Cell Biol.* **128,** 959–968.

Galceran, J., Farinas, I., Depew, M., Clevers, H., and Grosschedl, R. (1999). *Wnt3a*$^{-/-}$ like Phenotype and Limb Deficiency in *Lef1*$^{-/-}$ *Tcf1*$^{-/-}$ Mice. *Genes Dev.* **13,** 709–717.

Giese, K., Amsterdam, A., and Grosschedl, R. (1991). DNA-binding properties of the HMG domain of the lymphoid-specific transcriptional regulator LEF-1. *Genes Dev.* **5,** 2567–2578.

Giese, K., Cox, J., and Grosschedl, R. (1992). The HMG domain of lymphoid enhancer factor 1 bends DNA and facilitates assembly of functional nucleoprotein structures. *Cell* **69,** 185–195.

Giese, K., Kingsley, C., Kirshner, J., and Grosschedl, R. (1995). Assembly and function of a TCR alpha enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein–protein interactions. *Genes Dev.* **9,** 995–1008.

Graham, T. A., Weaver, C., Mao, F., Kimelman, D., and Xu, W. (2000). Crystal structure of a beta-catenin/Tcf complex. *Cell* **103,** 885–896.

Grosschedl, R., Giese, K., and Pagel, J. (1994). HMG domain proteins: Architectural elements in the assembly of nucleoprotein structures. *Trends Genet.* **10,** 94–100.

Harada, N., Tamai, Y., Ishikawa, T., Sauer, B., Takaku, K., Oshima, M., and Taketo, M. M. (1999). Intestinal polyposis in mice with

a dominant stable mutation of the beta-catenin gene. *EMBO J.* **18,** 5935–5942.

He, T. C., Chan, T. A., Vogelstein, B., and Kinzler, K. W. (1999). PPARdelta is an APC-regulated target of nonsteroidal anti-inflammatory drugs. *Cell* **99,** 335–345.

He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998). Identification of c-MYC as a target of the APC pathway [see comments]. *Science* **281,** 1509–1512.

He, X., Saint Jeannet, J. P., Woodgett, J. R., Varmus, H. E., and Dawid, I. B. (1995). Glycogen synthase kinase-3 and dorsoventral patterning in Xenopus embryos. *Nature* **374,** 617–622. [published erratum appears in *Nature* 1995 May 18: **375**(6528):253]

Heasman, J., Crawford, A., Goldstone, K., Garner Hamrick, P., Gumbiner, B., McCrea, P., Kintner, C., Noro, C. Y., and Wylie, C. (1994). Overexpression of cadherins and underexpression of beta-catenin inhibit dorsal mesoderm induction in early Xenopus embryos. *Cell* **79,** 791–803.

Hovanes, K., Li, T. W., Munguia, J. E., Truong, T., Milovanovic, T., Lawrence, M. J., Holcombe, R. F., and Waterman, M. L. (2001). beta-Catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. *Nat. Genet.* **28,** 53–57.

Huber, A. H., and Weis, W. I. (2001). The structure of the beta-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by beta-catenin. *Cell* **105,** 391–402.

Huber, O., Korn, R., McLaughlin, J., Ohsugi, M., Herrmann, B. G., and Kemler, R. (1996). Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. *Mech. Dev.* **59,** 3–10.

Ikeda, S., Kishida, S., Yamamoto, H., Murai, H., Koyama, S., and Kikuchi, A. (1998). Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. *EMBO J.* **17,** 1371–1384.

Ishitani, T., Ninomiya-Tsuji, J., Nagai, S., Nishita, M., Meneghini, M., Barker, N., Waterman, M., Bowerman, B., Clevers, H., Shibuya, H., and Matsumoto, K. (1999). The TAK1-NLK-MAPK-related pathway antagonizes signaling between beta-catenin and transcription factor TCF. *Nature* **399,** 798–802.

Kishida, S., Yamamoto, H., Ikeda, S., Kishida, M., Sakamoto, I., Koyama, S., and Kikuchi, A. (1998). Axin, a negative regulator of the wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of beta-catenin. *J. Biol. Chem.* **273,** 10823–10826.

Koh, T. J., Bulitta, C. J., Fleming, J. V., Dockray, G. J., Varro, A., and Wang, T. C. (2000). Gastrin is a target of the beta-catenin/TCF-4 growth-signaling pathway in a model of intestinal polyposis. *J. Clin. Invest.* **106,** 533–539.

Korinek, V., Barker, N., Willert, K., Molenaar, M., Roose, J., Wagenaar, G., Markman, M., Lamers, W., Destree, O., and Clevers, H. (1998a). Two members of the Tcf family implicated in Wnt/beta-catenin signaling during embryogenesis in the mouse. *Mol. Cell. Biol.* **18,** 1248–1256.

Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., Peters, P. J., and Clevers, H. (1998b). Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat. Genet.* **19,** 379–383.

Korinek, V., Barker, N., Morin, P. J., van Wichen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. (1997). Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC−/− colon carcinoma [see comments]. *Science* **275,** 1784–1787.

Korswagen, H. C., Herman, M. A., and Clevers, H. C. (2000). Distinct beta-catenins mediate adhesion and signalling functions in *C. elegans*. *Nature* **406,** 527–532.

Laudet, V., Stehelin, D., and Clevers, H. (1993). Ancestry and diversity of the HMG box superfamily. *Nucleic Acids Res.* **21,** 2493–2501.

Lin, R., Thompson, S., and Priess, J. R. (1995). pop-1 encodes an HMG box protein required for the specification of a mesoderm precursor in early *C. elegans* embryos. *Cell* **83,** 599–609.

Miller, J. R., Hocking, A. M., Brown, J. D., and Moon, R. T. (1999). Mechanism and function of signal transduction by the Wnt/beta-catenin and Wnt/Ca^{2+} pathways. *Oncogene* **18,** 7860–7872.

Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O., and Clevers, H. (1996). XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. *Cell* **86,** 391–399.

Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K. W. (1997). Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC [see comments]. *Science* **275,** 1787–1790.

Okamura, R. M., Sigvardsson, M., Galceran, J., Verbeek, S., Clevers, H., and Grosschedl, R. (1998). Redundant regulation of T cell differentiation and TCRalpha gene expression by the transcription factors LEF-1 and TCF-1. *Immunity* **8,** 11–20.

Oosterwegel, M., van de Wetering, M., Dooijes, D., Klomp, L., Winoto, A., Georgopoulos, K., Meijlink, F., and Clevers, H. (1991). Cloning of murine TCF-1, a T cell-specific transcription factor interacting with functional motifs in the CD3-epsilon and T cell receptor alpha enhancers. *J. Exp. Med.* **173,** 1133–1142.

Oosterwegel, M., van de Wetering, M., Timmerman, J., Kruisbeek, A., Destree, O., Meijlink, F., and Clevers, H. (1993). Differential expression of the HMG box factors TCF-1 and LEF-1 during murine embryogenesis. *Development* **118,** 439–448.

Orford, K., Crockett, C., Jensen, J. P., Weissman, A. M., and Byers, S. W. (1997). Serine phosphorylation-regulated ubiquitination and degradation of beta-catenin. *J. Biol. Chem.* **272,** 24735–24738.

Peifer, M., and Polakis, P. (2000). Wnt signaling in oncogenesis and embryogenesis: A look outside the nucleus. *Science* **287,** 1606–1609.

Pinson, K. I., Brennan, J., Monkley, S., Avery, B. J., and Skarnes, W. C. (2000). An LDL-receptor related protein mediates Wnt signalling in mice. *Nature* **407,** 535–538.

Polakis, P. (2000). Wnt signaling and cancer. *Genes Dev.* **14,** 1837–1851.

Polakis, P., Hart, M., and Rubinfeld, B. (1999). Defects in the regulation of beta-catenin in colorectal cancer. *Adv. Exp. Med. Biol.* **470,** 23–32.

Potten, C. S., and Loeffler, M. (1990). Stem cells: Attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development* **110,** 1001–1020.

Reya, T., O'Riordan, M., Okamura, R., Devaney, E., Willert, K., Nusse, R., and Grosschedl, R. (2000). Wnt signaling regulates B lymphocyte proliferation through a LEF-1 dependent mechanism. *Immunity* **13,** 15–24.

Riese, J., Yu, X., Munnerlyn, A., Eresh, S., Hsu, S. C., Grosschedl, R., and Bienz, M. (1997). LEF-1, a nuclear factor coordinating signaling inputs from wingless and decapentaplegic. *Cell* **88,** 777–787.

Rocheleau, C. E., Yasuda, J., Shin, T. H., Lin, R., Sawa, H., Okano, H., Priess, J. R., Davis, R. J., and Mello, C. C. (1999). WRM-1

activates the LIT-1 protein kinase to transduce anterior/posterior polarity signals in *C. elegans*. *Cell* **97,** 717–726.

Roose, J., Huls, G., van Beest, M., Moerer, P., van der, H. K., Goldschmeding, R., Logtenberg, T., and Clevers, H. (1999). Synergy between tumor suppressor APC and the beta-catenin-Tcf4 target Tcf1. *Science* **285,** 1923–1926.

Roose, J., Molenaar, M., Peterson, J., Hurenkamp, J., Brantjes, H., Moerer, P., van de Wetering, M., Destree, O., and Clevers, H. (1998). The Xenopus Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors. *Nature* **395,** 608–612.

Schilham, M., Wilson, A., Moerer, P., Benaissa-Trouw, B., Cumano, A., and Clevers, H. (1998). Critical involvement of Tcf-1 in expansion of thymocytes. *J. Immunol.* **161,** 3984–3991.

Schneider, S., Steinbeisser, H., Warga, R. M., and Hausen, P. (1996). Beta-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. *Mech. Dev.* **57,** 191–198.

Siegfried, E., Wilder, E. L., and Perrimon, N. (1994). Components of wingless signaling in Drosophila. *Nature* **367,** 76–80.

Takemaru, K. I., and Moon, R. T. (2000). The transcriptional coactivator CBP interacts with beta-catenin to activate gene expression. *J. Cell Biol.* **149,** 249–254.

Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J. P., and He, X. (2000). LDL-receptor-related proteins in Wnt signal transduction. *Nature* **407,** 530–535.

Tetsu, O., and McCormick, F. (1999). Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* **398,** 422–426.

Travis, A., Amsterdam, A., Belanger, C., and Grosschedl, R. (1991). LEF-1, a gene encoding a lymphoid-specific protein with an HMG domain, regulates T-cell receptor alpha enhancer function [corrected] *Genes Dev.* **5,** 880–894. [published erratum appears in *Genes Dev.* 1991 Jun; 5(6): following 1113].

van Beest, M., Dooijes, D., van de Wetering, M., Kjaerulff, S., Bonvin, A., Nielsen, O., and Clevers, H. (2000). Sequence-specific high mobility group box factors recognize 10-12-base pair minor groove motifs. *J. Biol. Chem.* **275,** 27266–27273.

van de Wetering, M., Oosterwegel, M., Dooijes, D., and Clevers, H. (1991). Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box. *EMBO J.* **10,** 123–132.

van de Wetering, M., Castrop, J., Korinek, V., and Clevers, H. (1996). Extensive alternative splicing and dual promoter usage generate Tcf-1 protein isoforms with differential transcription control properties. *Mol. Cell. Biol.* **16,** 745–752.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., Peifer, M., Mortin, M., and Clevers, H. (1997). Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. *Cell* **88,** 789–799.

van Genderen, C., Okamura, R. M., Farinas, I., Quo, R. G., Parslow, T. G., Bruhn, L., and Grosschedl, R. (1994). Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev.* **8,** 2691–2703.

Verbeek, S., Izon, D., Hofhuis, F., Robanus Maandag, E., te Riele, H., van de Wetering, M., Oosterwegel, M., Wilson, A., MacDonald, H. R., and Clevers, H. (1995). An HMG-box-containing T-cell factor required for thymocyte differentiation. *Nature* **374,** 70–74.

von Kries, J. P., Winbeck, G., Asbrand, C., Schwarz-Romond, T., Sochnikova, N., Dell'Oro, A., Behrens, J., and Birchmeier, W. (2000). Hot spots in β-catenin for interactions with LEF-1, conduction and APC. *Nat. Struct. Biol.* **7,** 800–807.

Waltzer, L., and Bienz, M. (1998). *Drosophila* CBP represses the transcription factor tcf to antagonize Wingless signaling. *Nature* **395,** 521–525.

Waterman, M. L., Fischer, W. H., and Jones, K. A. (1991). A thymus-specific member of the HMG protein family regulates the human T cell receptor C alpha enhancer. *Genes Dev.* **5,** 656–669.

Webster, M. T., Rozycka, M., Sara, E., Davis, E., Smalley, M., Young, N., Dale, T. C., and Wooster, R. (2000). Sequence variants of the axin gene in breast, colon, and other cancers: An analysis of mutations that interfere with GSK3 binding. *Genes Chromosomes Cancer* **28,** 443–453.

Wehrli, M., Dougan, S. T., Caldwell, K., O'Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Schejter, E., Tomlinson, A., and DiNardo, S. (2000). arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* **407,** 527–530.

Wodarz, A., and Nusse, R. (1998). Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **14,** 59–88.

Yost, C., Torres, M., Miller, J. R., Huang, E., Kimelman, D., and Moon, R. T. (1996). The axis-inducing activity, stability, and subcellular distribution of beta-catenin is regulated in Xenopus embryos by glycogen synthase kinase 3. *Genes Dev.* **10,** 1443–1454.

Zhou, P., Byrne, C., Jacobs, J., and Fuchs, E. (1995). Lymphoid enhancer factor 1 directs hair follicle patterning and epithelial cell fate. *Genes Dev.* **9,** 700–713.

Received for publication June 25, 2001
Revised November 28, 2001
Accepted November 28, 2001
Published online February 11, 2002